Directorate General of Health Services
Ministry of Health & Family Welfare
Government of India

# Manual on Laboratory Procedures of Common Epidemic-Prone Diseases

## MEASLES

National Institute of Communicable Diseases
22 Shamnath Marg, Delhi-110 054
1984

COMMUNITY HEALTH CELL

# Manual On Laboratory Procedures Of Common Epidemic-Prone Diseases

## MEASLES

Editors

R. N. Basu, M.B.,B.S., D.P.H., M.S. (Harvard)

Rajesh Bhatia, M.B.,B.S., M.D.

R. L. Ichhpujani, B.Sc, M.B.,B.S., M.D.

Directorate General of Health Services,
National Institute of Communicable Diseases,
22, Shamnath Marg, Delhi-110054
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# Preface

Communicable diseases constitute a single most important cause for high morbidity and mortality in all age groups in developing countries. Most of these diseases can be easily prevented or cured at an early date if timely intervention in the form of investigation of suspected outbreak, confirmation of diagnosis and containment activities are provided. The initiation of specific measures is based on the rapid and accurate diagnosis of the prevalent disease. The laboratory has thus an important role to play. While there is no denying the fact that the most sensitive and reliable tests should be used in the laboratory, yet it is easily understandable that the final outcome shall depend upon the type and quality of the morbid material received at the laboratory for a particular test. The knowledge of the type of diagnostic material to be collected, how and when to collect and how and where to transport is absolutely essential for arriving at an accurate diagnosis.

Experience has shown that this basic and perhaps the most vital knowledge is lacking at the periphery and regional levels. No wonder, many disease outbreaks remain undiagnosed and misappropriately labelled as 'mysterious diseases'. A need had been felt in different quarters regarding the availability of a compact manual encompassing various aspects of the diagnosis. The National Institute of Communicable Diseases (NICD), Delhi took the initiative and with the technical and financial assistance from World Health Organization (WHO) organised the first 'NICD/WHO Workshop on Preparation of a Manual on Laboratory Procedures of Common Epidemic-Prone Diseases' at the NICD campus from 2nd to 5th May, 1984. On the basis of current morbidity and mortality figures and the fact that state health authorities are commonly confronted with the investigations of these five diseases viz. poliomyelitis, measles, Japanese encephalitis, diarrhoeal diseases and viral hepatitis were selected. There are at present only few laboratories competent to undertake various tests to confirm the diagnosis of Measles.

There is need for uniformity in testing procedure and interpretation of results.

In addition to strengthening the hospital services, this manual is meant to provide laboratory support to the epidemiological investigation of outbreaks of measles, in the various districts of states and even in other neighbouring states. It is expected that this will be the link between the microbiologist in the laboratories and the public health physicians in the field.

Well before the commencement of the Workshop, leading institutions in India were requested to submit a draft manual, or base paper on the disease in which the particular institute specialised. On measles a draft manual prepared by Dr. S. M. Saha was sent by Dr. S. N. Saxena, Director, Central Research Institute, Kasauli. Faculty of NICD, Delhi also prepared a draft manual on this disease. Dr. (Mrs.) Shashi Khare and Dr. C. L. Sehgal were mainly instrumental in giving shape to NICD draft manual.

The participants in this Workshop drew heavily from these two draft documents to give final shape to a manual which was unanimously adopted by the group on measles. For making the manual compact and workable, only those tests which are practical and feasible under the present set up have been included. Unnecessary theoretical details have been deliberately avoided. The names of important laboratories working on measles have been appended so that referral services can easily be sought. A small chapter on the safety in the laboratory has also been included.

It was rightly and repeatedly emphasized during the Workshop that mere publication of the manual should not be the culmination of all the hard work that has gone into it. To make this document successfully operative in the field is more important. In this respect, NICD is willing to assist any individual or organisation in all possible ways.

We are grateful to Dr. D. B. Bisht, Director General of Health Services, for his continous support and guidance in conducting this workshop and publication of the manual.

We place on record our appreciations for all those who pooled their efforts, either before or during the workshop in preparing this manual. The editorial trimmings might have inadvertently resulted into some mistakes, for which we own the responsibility. Any criticism and suggestions for improvement of subsequent editions of manual are most welcome and shall be duly acknowledged.

R. N. Basu
Rajesh Bhatia
R. L. Ichhpujani

Measles is an acute, highly infectious viral disease characterized by maculopapular rash, fever, respiratory symptoms and Koplik's spots on the buccal mucosa. It is commonly known as 'Khasra'.

## 1. 1 Aetiological Agent :

The aetiological agent is the measles virus which is a member of the genus *Morbilli virus* of the family *Paramyxoviridae.*

## 1. 2 Mode of Transmission :

Infection is contracted by inhalation of droplets expelled in sneezing or coughing. Measles is spread chiefly by children during catarrhal prodormal period. Patients are infectious from 1-2 days prior to the onset of symptoms until a few days after the rash has appeared. Measles is one of the most readily transmitted communicable diseases. The only reservoir of infection is man.

## 1. 3 Incubation Period :

It is about 10 days to the onset of fever and 14 days to the appearance of rash.

## 1. 4 Clinical Features :

The disease is characterised by prodromal fever, conjunctivitis, coryza, bronchitis and Koplik's spots on the buccal mucosa. A characteristic red blotchy rash appears on the 3rd or 4th day beginning on the face, becoming generalized and lasting 4 to 7 days and sometimes ending in branny desquamation.

This disease often leads to complications which include secondary bacterial infections resulting into otitis media, pneumonia and encephalitis.

Measles can be easily diagnosed clinically. About five percent of cases lack Koplik's spots and are difficult to differentiate clinically from infection with rubella virus, certain enteroviruses and adenoviruses.

## 1. 5 Incidence in India :

Measles is endemic throughout the world. In general, epidemics occur regularly every 2 to 3 years. The disease flares up when there is

an accumulation of susceptible children. By age of twenty years, over 80 percent have had an attack of the disease. Only about one percent of susceptible persons fail to develop measles on their first close contact with a patient. The highest incidence of measles is in late winter and spring. It is estimated that 80-90 percent of the children suffer from this disease in India.

It is an important public health problem which leads to malnutrition, secondary infection and death in few children. In India, overall case fatality rate ranges between 0. 26 to 3. 50 percent. The mortality is mainly due to secondary bacterial infections.

## 2. Collection, Storage and Transportation of Specimens

Samples can be collected for the isolation of virus and for demonstration of specific measles antibodies.

### 2. 1 Specimen for the Isolation of Virus :

**2. 1. 1 Blood :**

Draw 5 ml of blood into a sterile syringe and transfer immediately into a sterile screw capped bottle.

**2. 1. 2 Throat Secretions :**

i *Collection from Adults :* Instruct the patient to gargle with 15 ml of sterile Earle's fluid (annexure 7. 2. 1. 3) containing 0. 5 percent gelatin and to expectorate the contents of the mouth in sterile wide mouth screw capped bottle. Add 500 $\mu$g per ml of streptomycin and 500 units of penicillin per ml of the Earle's fluid and immediately transport to the laboratory on ice.

ii *Collection from Children :* Collect the throat secretions by gently swabbing the tonsillar and pharyngeal areas with sterile cotton tipped swab. Place it in a sterile screw capped bottle containing 3 ml of Earle's fluid, containing antibiotics as mentioned above.

In case the above samples cannot be sent immediately to the laboratory for virus isolation, keep these at $+4°C$ for not more than 24 hours.

**2. 1. 3 Transportation of Specimens :**

Properly label the specimen bottles and securely pack these in cellophane bags with rubber bands to prevent seepage of ice water. Keep packed specimen in a thermocole box or thermos with ice and send to the nearest laboratory (Chapter 8) through a special messenger.

### 2. 2 Specimen for Serological Studies :

Serology reveals valuable information on the past incidence of measles in the subjects and in the community, in diagnosis of suspected measles infection and in evaluation of vaccination by studying sero-conversion. Except in the epidemiological study on the past incidence

of measles in the community, paired samples of sera are essential for serology. An acute phase and a convalescent phase serum of the patient are the pre-requisite for the retrospective diagnosis of measles infection. Pre and post vaccination sera are essential to study seroconversion after measles immunisation.

**2. 2. 1 Serum :**

i Collect 2 ml of blood in a sterile syringe under all aseptic precautions and transfer to a sterile screw capped bottle. Allow the blood to clot at room temperature and then keep overnight in refrigerator (+4°C). Separate the serum and store in the refrigerator (+4°C) for not more than 2 days before sending it to the laboratory.

ii Blood samples can also be collected by finger prick method on filter paper strips (Whatman filter paper No. 1) inscribed with a circle of 14 mm diameter. Saturate the inscribed circle with the blood and allow it to dry without folding. Mark strip serially and keep these in cellophane bag.

Screw capped bottles containing serum samples securely packed and labelled as well as blood samples collected on filter strips in cellophane bags may be sent to the testing laboratory by post.

All specimens must be properly labelled with name, serial No., age, sex and date of collection and should be accompanied by the pertinent information in the proforma (annexure 7.1).

## 3. Laboratory Diagnosis

Of the two major components of the laboratory diagnosis viz. isolation of the virus and the serological tests, the former is done only under special circumstances and tried only when other tests are not conclusive. Serology plays the all important role. Of the various tests microhaemagglutination inhibition test and *in vitro* neutralization test have been shown to be very sensitive as well as specific and hence are recommended.

### 3.1 Serological Tests :

#### 3.1.1 **Microhaemagglutination Inhibition Test :**

##### *3.1.1.1 PRINCIPLE :*

A wide variety of animal viruses have been shown to possess the capacity of adsorbing to red blood cells and in many cases causing their agglutination. Virus haemagglutination is readily inhibited by specific antibody and the haemagglutination inhibition test furnishes a sensitive method for detecting viral antibodies.

##### *3.1.1.2 EQUIPMENT AND REAGENTS :*

i Measles haemagglutination (HA) antigen (available from Central Research Institute, Kasauli)

ii Rhesus monkey erythrocytes (available from Central Research Institute, Kasauli, and N.I.C.D., Delhi)

iii Physiological saline, pH 7.2 (annexure 7.2.1.2)

iv Alsever's solution (annexure 7.2.1.1)

v Known positive and negative measles antisera (available from Central Research Institute, Kasauli)

vi Graduated centrifuge tube (15 ml capacity)

vii Plastic plates with 'U' or 'V' shaped wells with lids

viii Microdiluters (25 $\mu$l)

ix Micro-droppers (25 $\mu$l and 50 $\mu$l)

x Mirror for reading the plates

*3.1.1.3 PROCEDURE :*

3.1.1.3.1 COLLECTION OF MONKEY BLOOD :

Collect rhesus monkey blood under sterile conditions in Alsever's solution in the ratio of 1 in 4 and distribute the preserved blood in small aliquotes and store at+4°C. These cells can be used upto 10 days.

3.1.1.3.2 WASHING OF ERYTHROCYTES :

i Centrifuge the required quantity of the preserved blood in graduated centrifuge tube for 10 minutes at 2000 rpm.

ii Aspirate off the supernatant fluid and buffy layer of WBC with pasteur pipette attached to a rubber bulb without disturbing the packed RBC.

iii Add 2 ml of physiological saline.

iv Mix gently with pasteur pipette to resuspend the cells and add additional quantity of physiological saline to bring it to the original volume.

v Centrifuge at room temperature for 10 minutes at 2000 rpm.

vi Repeat three times the steps ii to v.

vii Centrifuge at the end for 10 minutes at 2000 rpm and discard the supernatant.

viii Read volume of packed RBC.

ix Add physiological saline solution to make 10 percent suspension of RBC. For use in the test prepare O. 5 percent suspension of RBC in physiological saline.

3.1.1.3.3 TREATMENT OF SERA :

i Prior to day of test punch out the blood impregnated discs of filter paper measuring 10 mm in diameter with a metal punch or cut out by scissors.

ii Place the disc in a test tube (13 mmx85 mm). Add 0.2 ml of physiological saline and keep at room temperature for 30 minutes with intermittent shaking.

iii Remove the filter paper disc with a metal rod after expressing the fluid. The elute will represent 1:16 dilution of the serum.

iv Inactivate the elute or the serum sample at 56°C for 30 minutes.

v Adsorb the inactivated sera overnight in a refrigerator with 50 $\mu$l of 50 percent rhesus monkey erythrocytes.

3.1.1.3.4 TITRATION OF ANTIGEN :

i With a wax pencil, mark a 'U' microtitre plate as shown below :

Antigen dilution : 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and cc (cell control).

ii With 25$\mu$l dropper add one drop of physiological saline in all the wells.

iii Add 25 $\mu$l of measles antigen in first well and make doubling dilution with the help of microdiluters.

iv With 25 $\mu$l dropper add one drop more of saline into each well.

v Add 50 $\mu$l volume of 0.5 percent normal rhesus monkey RBC suspsnsion to each well with the help of micro-dropper.

vi Tap the plate or use shaker to mix cells and antigen dilutions.

vii Cover the plate and incubate at 37°C for 1-2 hours.

viii Read and record the titration.

3.1.1.3.5 INTERPRETATION :

Complete agglutination consists of a layer of uniformly agglutinated cells covering the bottom of well.

A negative pattern consists of a compact sharply demarcated button of sedimented cells in the centre of the bottom of the well, similar to that seen in the central wells. The results can also be read by tilting the plate and looking for the free flow of cells indicating absence of agglutination. The highest dilution of antigen with complete agglutination is the end point and contains one haemagglutination (HA) unit e.g. if HA result is as follows :

| 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|---|---|---|---|---|---|---|---|
| + | + | + | + | + | + | — | — |

Antigen titre is 1:64 which contains 1 HA unit.

3.1.1.3.6 PROCEDURE FOR HAEMAGGLUTINATION INHIBITION (HI) :

i Use the above titrated antigen for the sera samples.

ii Treat all the test sera and known positive and negative control sera as described under 3.1.1.3.3.

iii Mark the microtitre plate to accommodate the number of sera to be tested for each antigen. One pattern is shown below :

| | Test serum dilutions | | | | | | Control serum | |
|---|---|---|---|---|---|---|---|---|
| Sr. No. | 1:16* | 32 | 64 | 128 | 256 | 512 | 1:16 | 1:32 |
| 1. | | | | | | | | |
| 2. | | | | | | | | |
| 3. | | | | | | | | |
| 4. | | | | | | | | |
| 5. | | | | | | | | |
| 6. | | | | | | | | |
| 7. | | | | | | | | |
| 8. | | | | | | | | |
| 9. | | | | | | | | |
| 10. | | | | | | | | |
| 11. | | | | | | | | |
| 12. | | | | | | | | |

*Neat serum can also be used with a starting dilution of 1:2,

iv With the help of 25 $\mu$l dropper pipette put 25 $\mu$l of physiolgical saline in all the wells except first and seventh wells.

v Add 25 $\mu$l of 1 : 16 dilution of sera to first, second and seventh wells.

vi Make doubling dilution of sera from 1 : 32 to 1 : 512.

vii The last two wells shall have control serum dilutions of 1:16 and 1:32.

viii Add 25 $\mu$l of measles antigen having 4 HA units from first to sixth well of each row (if titre is 1 : 64 which is equivalent to one HA unit then for 4 HA units, antigen has to be diluted 1 : 16 for using in the test).

ix Add 25 $\mu$l of physiological saline in seventh and eighth wells. Keep the plate at room temperature.

x Add 50 $\mu$l of 0. 5 percent normal monkey erythrocytes to all the wells with the help of dropper.

xi Mix the contents either by tapping or on a shaker. Cover with lid.

xii Incubate at 37°C for 1$\frac{1}{2}$ to 2 hours.

xiii With each test put up positive serum control, cell control and antigen control to check the efficacy of test.

xiv Read and record the cell patterns :-

a Check RBC controls. These should settle down as a button.

b Check the titration of antigens. Antigen should contain 4 HA units.

c Check the serum controls. There should be no haemagglutination.

d Positive control serum should give known HI titre.

e Check the test sera. The end-point designates the titre of the antibody viz. the highest dilution of serum that inhibits haemagglutination.

3. 1. 1. 3. 7 INTERPRETATION :

HI titre of 1 : 4 or more is of diagnostic significance. In this respect the filter paper technique has the disadvantage that its starting dilution is 1 : 16. Otherwise this is a simple and rapid test albeit less sensitive than the neutralization test.

**3. 1. 2 Neutralization Test** (*in vitro*) :

*3. 1. 2. 1 PRINCIPLE :*

Serum specimens are assayed for neutralizing antibody content by testing serial dilutions of the serum against a standard dose (100 $TCID_{50}$) of the virus by the constant virus-varying serum technique.

*3.1.2.2 EQUIPMENT AND REAGENTS* :

i Vero cell culture

ii Edmonston strain of measles virus (may be obtained from Division of Biologic Standards, National Institute of Health, U. S. P. H. S., Betheseda, Maryland, USA). If other strains are to be employed then it is recommended that their infectivity titres be equivalent to those of the standard virus under the same cell culture system

iii Known positive measles serum

iv Automatic 1 ml syringe to deliver 0.1 ml with supply of sterile needles or disposable tips

v Inverted microscope or microscope with improvised test tube stand

vi Racks for loading tissue culture tubes in slanting position

vii Earle's solution (annexure 7. 2. 1. 3)

viii Growth medium (annexure 7. 2. 1. 4)

ix Maintenance medium (annexure 7. 2. 1. 5)

*3. 1. 2. 3 VERO CELL CULTURES FOR ASSAY IN TUBES* :

i Prior to assays, cells from an ampoule are reconstituted in an 40 $cm^2$ MD bottle with 15 ml of growth medium.

ii Replace the growth medium after overnight incubation to remove dimethylsulphoxide.

iii Resuspend the cells after formation of confluent monolayer and subculture in a 190 $cm^2$ Roux bottle with 90 ml of growth medium.

iv Resuspend the cells from 4-5 days confluent cultures in growth medium and adjust the concentration to $5.7 \times 10^5$ cells per ml. For each batch of assays, prepare a single homogenous pool of cells.

v Dispense the cell suspension into tubes in 1 ml aliquots and close tightly with rubber stoppers.

vi Maintain the tubes in an inclined position to obtain a maximum confluent monolayer within 2-3 days.

3.1.2.3.1 TITRATION OF VIRUS IN TUBES :

i Prepare stock suspension of virus in the form of infected tissue culture fluid and store at -70°C.

ii From stock virus make 10 fold dilutions viz. 1 : 10, 1 : $10^2$, .....1 : $10^8$ using maintenance medium.

iii Remove the growth medium from the cell culture tubes showing complete sheet under microscope. Label these and put the mark on ths side opposite cell sheet.

iv Inoculate 0. 1 ml of each dilution of virus to 5 cell culture tubes and place in an incubator at 37°C in slanting position.

v Put 1 ml of maintenance medium with the help of automatic syringe to each tube.

vi Incubate in slanting position at 35°C for fourteen days.

vii Observe daily for typical cytopathic effect (CPE) like giant cell formation.

3.1.2.3.2 CALCULATION OF ANTIGEN :

Calculate $TCID_{50}$ per 0.1 ml on the basis of CPE by Reed and Meunch or Karber methods and use 100 $TCID_{50}$ per 0.1 ml for the actual test.

3.1.2.3.3 TEST PROCEDURE :

i Dilute stock virus in maintenance medium so as to have 100 $TCID_{50}$ per 0.1 ml.

ii Make doubling dilution of serum viz 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. (Elute from filter paper strips cannot be used).

iii Mix 0.5 ml of virus with 0.5 ml of each dilution of serum.

iv Keep serum virus mixture at +4°C for 1 hour.

v Add 0.2 ml of each serum virus mixture to 3 cell culture tubes showing complete sheet.

vi For infectivity control, inoculate three sets of cell culture tubes each with 0.1 ml of virus dilutions of 100, 10 and 1 $TCID_{50}$ respectively per 0.1 ml.

vii Incubate at 37°C for 1 hour.

viii Add 1 ml of maintenance medium in all the tubes.

ix Put cell controls with each test.

x Incubate at 35°C in slanting position for fourteen days.

xi Observe daily for CPE.

3.1.2.3.4 INTERPRETATION :

The highest dilution of serum which shows absence of CPE is titre of antibody. A four-fold rise in the paired serum samples is considered diagnostic.

Note : An undiluted serum sample should also be tested since, occasionally even neutralization titres decline to very low levels especially many years after an attack of measles. Very rarely neutralising antibody may not be present to a detectable level in persons immune to measles.

*3.1.2.4 VERO CELL CULTURES FOR ASSAY BY MICRO-TECHNIQUE :*

i Resuspend in growth medium a single homogenous pool of cells obtained from 5-8 days confluent cultures.

ii Adjust concentration to $5x10^5$ cells per ml on the basis of haemocytometer count.

iii Dispense 50 $\mu$l aliquots of cell suspension into each of 10 wells and the 12th wells of a 96 well (8x12) microtitre tissue culture plate.

iv Cover the plate with an adhesive cellophane tape and incubate at 37°C for 4 hours.

3.1.2.4.1 TITRATION OF VIRUS IN MICRO PLATES :

i Prepare serial four-fold dilutions of virus in chilled maintenance medium. (The test dilution range should cover 100 percent CPE to 100 percent non-CPE).

ii Remove the adhesive tape and add 0.2 ml aliquots of the test virus dilutions to each of 10 wells of microtitre plate.

iii Add 0.2 ml aliquots of diluent to 12th well of the plate to serve as cell control.

iv Cover the plate with an adhesive cellophane tape.

v Incubate the plates at 35°C for 14 days.

vi Observe the entire surface of each well for measles specific CPE on an inverted microscope.

vii Calculate the titre as per tube technique.

Note : In micro technique all dilutions and dispensing of small volumes are carried out using fixed volume micro-pipettes with disposable polypropylene tips.

3.1.2.4.2 PROCEDURE OF TEST :

i Prepare the Vero cell culture in microplate as already mentioned.

ii Make doubling dilutions of serum in tubes from 1:4 to 1:512.

iii Mix 0.5 ml 100 $TCID_{50}$ per 0.1 ml of virus with 0.5 ml of each serum dilution.

vi Keep serum virus mixture at + 4°C for 1 hour.

v Add 0.2 ml of each serum virus mixture into 4 wells of microplate.

vi For infectivity control inoculate each of 10 wells having cell culture sheet with 0. 2 ml of virus dilutions containing 100, 10 and 1 $TCID_{50}$ respectively per 0. 2 ml.

vii Put cell controls with each test.

viii Cover the plates with adhesive tape and incubate at 35°C for 10 days.

ix Observe daily for CPE.

## 3. 2 Isolation of Virus :

Measles virus can be isolated from naso-pharyngeal secretions or blood using Vero cells. Necessary biosafety procedures should be observed in virological work.

### 3. 2. 1 Equipment and Reagents :

i Vero cell cultures

ii Growth medium and maintenance medium (annexure 7. 2. 1. 4)

iii Inverted microscope or microscope with improvised test tube holding stand

iv Refrigerated centrifuge

v Low temperature cabinet (-70°C)

### 3. 2. 2 Processing of Specimen :

i In case of throat swab squeeze it with a dry sterile forcep in the medium.

ii Centrifuge all the three types of specimens i.e. throat swab, nasopharyngeal secretions and blood at 5000 rpm for 15 minutes at +4°C.

iii Remove the supernate containing the virus.

iv If the delay is inevitable, store the material at -70°C.

### 3. 2. 3 Procedure :

i Prepare monolayer cell cultures in test tubes as described under 3. 1. 2. 3.

ii On the day of inoculation, examine the cultures microscopically to ensure that complete monolayer has formed.

iii Discard the growth medium.

iv Inoculate 0. 2 ml of processed sample in each of three cell culture tubes.

v Incubate tubes at 37°C in horizontal position for one hour.

vi Add 1 ml of maintenance medium.

vii Put up cell controls.

viii Incubate at 35°C in an incubator in slanting position.

ix Observe daily for CPE upto 30 days. Change the medium twice a week. CPE usually develops within 5-10 days with wild virus.

x Perform one blind passage with negative culture on 30th day after inoculation. This may help in detecting a positive specimen having a low titre of virus.

xi Harvest the fluid from cultures showing CPE and store at -70°C.

xii Use the cells showing CPE for haemadsorption test.

**3. 2. 4 Haemadsorption test for Identification :**

i Carry out haemadsorption test by adding 0. 5 percent monkey erythrocyte suspension in suspected cultures and control tubes.

ii Rinse the cultures with Earle's fluid after 30 minutes.

iii Discard the medium and examine under microscope. The RBCs will adhere to the area of cells showing CPE. No adsorption of RBCs will be observed in control cell cultures.

## 4. Retrieval of Measles Vaccine for Potency Testing

Pick up at random the measles vaccine vials from the field and transport to the laboratory on ice for testing the potency. The retrieved vaccine should be sent to C. R. I., Kasauli (Himachal Pradesh). It should be either air freighted with prior intimation to C. R. I. or sent through a courier who should replenish the ice as and when required. The reconstituted vaccine is NOT suitable for potency testing.

## 5. Safety Precautions In The Laboratory

Biosafety in a microbiological laboratory is very essential and basically depends on three components :

a Basic standard of laboratory design, operation and equipment.

b Selection and use of essential biosafety equipment.

c Safe laboratory procedures.

An exhausative review of each component is beyond the scope of this manual but practical and easily achievable safe laboratory rules are listed here.

i Avoid mouth pipetting.

ii Avoid eating, drinking, smoking and storing eatables in the laboratory.

iii Decontaminate the working area atleast once a day and more frequently after the spillage of potentially infective material.

iv Wash your hands after handling the infectious material.

v Wear laboratory coats/gowns in the laboratory and these should not be taken outside.

vi Use gloves for all those procedures that may involve accidental direct contact with blood or infectious materials.

vii Decontaminate all liquid or solid waste before disposal.

viii Perform all technical procedures in a way that minimises the aerosol formation.

ix Provide adequate training to the staff in laboratory safety procedures.

x As far as possible actively immunize the workers against the diseases the materials of which they are handling.

xi Employ only medically fit staff to work in clinical laboratories.

xii Report accident and illness promptly to the concerned officials.

xiii Provide ample space and illumination for safe conduction of laboratory procedures.

xiv Design smooth, easily cleanable walls, ceilings and floors which should be impermeable to liquids and resistant to chemicals and disinfectants. Floors should be slip-resistant.

xv Ensure a dependable and good quality water supply.

xvi Make suitably equipped first aid rooms readily accessible.

xvii Provide the staff safe laboratory equipments e.g. pipetting aids, safety cabinets, screw cap tubes and bottles, loop micro incinerators and autoclaves etc.

xviii Carry out periodic health and medical surveillance of the workers to exclude the highly susceptible individuals.

xix Provide safety systems covering fire and electrical emergencies.

xx Control rodents and insects in the laboratory.

xxi Don't permit the entry of the experimental animals, which are not to be used in the laboratory.

## 6. Further Reading


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## 7. Annexures

### 7.1 Proforma to Accompany Specimens :

Sr. No.....

Name of tha Patient.....
Father's Name.....
Age..... Sex.....
Full Address.....
.....
Date of onset.....
Date of appearance of rash, if any.....
CLINICAL SYMPTOMS :
Tick as indicated.

Fever ☐ Conjunctivitis ☐ Coryza ☐

Koplik's spot ☐ Bronchitis ☐ Red blochy rash ☐

Others (Specify).....

History of measles immunization : Yes ☐ No ☐

SAMPLES COLLECTED :

| Nature | Date of collection | Date of despatch |
|---|---|---|
| Blood | | |
| Throat secretions | | |
| Throat Swab | | |
| Serum samples | Acute ☐ | |
| | Convalescent ☐ | |
| Filter paper strips | | |
| | Prevaccination ☐ | |
| | Post vaccination ☐ | |

Signatures

## 7. 2 Preparation of Reagents :

### 7. 2. 1 Reagents for Haemagglutination Inhibition :

*7. 2. 1. 1 ALSEVER'S SOLUTION :*

| | | | |
|---|---|---|---|
| Dextrose | ... | ... | 20. 500 gm |
| NaCl | ... | ... | 4. 200 gm |
| Sodium citrate | ... | ... | 8. 000 gm |
| Citric acid | ... | ... | 0. 550 gm |

Add double distilled water to make it to 1000 ml. Autoclave at 10 lbs for 10 minutes.

*7. 2. 1. 2 PHYSIOLOGICAL SALINE (PH 7. 2)* :

Add 9 gm NaCl to 1000 ml of distilled water. Autoclave at 15 lbs for 15 minutes. Adjust to pH 7. 2

*7. 2. 1. 3 EARLE'S 10 x SOLUTION :*

| | | | |
|---|---|---|---|
| NaCl | ... | ... | 68. 0 gm |
| KCl | ... | ... | 4. 0 gm |
| $CaCl_2$ | ... | ... | 2. 0 gm |
| $MgSO_4. 7H_2O$ | ... | ... | 2. 0 gm |
| $NaH_2 PO_4. H_2O$ | ... | ... | 1. 4 gm |
| Phenol red | ... | ... | 0. 2 gm |
| Double glass distilled or deionised water | ... | ... | 1000 ml |
| Chloroform | ... | ... | 2 ml |

Dissolve the ingredients separately. Keep at +4°C before use.

For preparation of Earle's 1x solution, take 100 ml of Earle's 10x solution and make up the volume to 1 litre with sterile double glass distilled or deionised water. Autoclave at 15 lbs for 20 minutes.

*7. 2. 1. 4 GROWTH MEDIUM* :

It consists of Earle's Minimum Essential Medium (MEM) with non-essential amino acids in Earle's Salts, pH 7. 4, supplemented with 10 percent calf or sheep serum and antibiotics.

*7. 2. 1. 5 MAINTENANCE MEDIUM :*

MEM supplemented with 2 percent calf or sheep serum and antibiotics.

*7. 2. 1. 6 PENICILLIN AND STREPTOMYCIN SOLUTION :*

Take benzyl penicillin (5 lakhs I. U. per vial) and streptomycin sulphate (1 gm vial). Put 1 ml Earle's solution in each of 4 vials of penicillin (20 lakhs units) and in 2 vials of streptomycin (2 gm). Pool their contents in the flask. Make it to 50 ml with Earle's solution and filter through millipore or sintered glass filter. Thus 1 ml of antibiotics solution contains 40,000 units of penicillin and 40 mgm of streptomycin. Of this 0. 25 ml is added to 100 ml of medium. Thus 1 ml of medium will have 100 units of penicillin and 100 $\mu$g of streptomycin.

*7. 2. 1. 7. MYCOSTATIN SOLUTION :*

Take one vial having 500,000 units. Suspend the contents of the vial in 4 ml of absolute alcohol and finally make upto 20 ml with sterile distilled water. Add 0. 1 ml of this to 100 ml of medium. The final concentration will be 25 units per ml.

OR

*FUNGIZONE SOLUTION :*

Dissolve 50 mgm in 100 ml of distilled water and filter as above. Add 0. 5 ml to 100 ml of medium. The final concentration is 2. 5 mg per ml.

## 7. 3 Washing of Glassware :

**7. 3. 1. New Glassware :**

i Put glassware in boiling Lomosol or detergent surf solution (3 oz in 1 gallon of water) for 1 hour.

ii Rinse, brush and wash well several times with tap water.

iii Keep overnight in 10 percent HCl.

iv Wash thoroughly with tap water and keep overnight in demineralised or distilled water.

v Wash three times in demineralised or distilled water.

vi Dry at 70°C in hot air oven.

vii Plug the flasks, pipettes and tubes etc. with cotton gauze.

viii Sterilize in hot air oven at 160°C for 2 hours.

**7. 3. 2 Used Glassware :**

i Fill with water and decontaminate in autoclave at 121°C for 20 minutes.

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ii Put in detergent solution, rinse, brush and wash thoroughly with tap water.

iii Follow the procedure from iv to viii as described under 7. 3. 1.

**7. 3. 3 New Rubber Corks :**

i Dissolve 20 gm NaOH in one litre distilled water.

ii Boil corks in it for 30 minutes.

iii Wash thoroughly and soak overnight in demineralised or distilled water

iv Dry and autoclave.

## 8. Laboratories Undertaking Work on Measles

i Central Research Institute,
Kasauli. (H. P.)

ii Postgraduate Institute of
Medical Education and Research,
Chandigarh.

iii All India Institute of
Medical Sciences,
Ansari Nagar,
New Delhi.

iv National Institute of
Communicable Diseases,
22, Sham Nath Marg,
Delhi.

v Christian Medical College,
Vellore (Tamil Nadu).

vi Haffkine Institute,
Parel,
Bombay.

## 9. Members of the Group on Measles in the Workshop on Preparation of a Manual on Laboratory Procedures of Common Epidemic-Prone Diseases Held at NICD Delhi From 2nd to 5th May, 1984

i Prof. C. S. Lakshminarayan, — Chairman
Medical College,
Madras.

ii Sh. S. M. Saha, — Rapporteur
Central Research Institute,
Kasauli. (H. P.)

iii Dr. B. B. Gaitonde,
WHO-SEARO,
Indraprastha Estate,
New Delhi.

iv Dr. K. K. Datta,
D. G. H. S.,
New Delhi.

v Dr. Y. I. Singh,
Regional Medical College,
Imphal.

vi Dr. R. N. Basu,
National Institute of Communicable Diseases,
22, Shamnath Marg,
Delhi.

vii Dr. C. L. Sehgal,
National Institute of Communicable Diseases,
22-Sham Nath Marg,
Delhi.

viii Dr. T. K. Ghosh,
National Institute of Communicable Diseases,
22-Sham Nath Marg,
Delhi.

# NOTES

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Many disease outbreaks remain undiagnosed and misappropriately labelled as "mysterious" due to the lack of basic knowledge regarding the type of diagnostic material to be collected, how and when to collect, and how and where to transport it and the possible tests that can provide an accurate and reliable diagnosis. This manual is designed to bridge this gap in the existing knowledge.

Other manuals available in this series are :

* Viral Hepatitis
* Japanese Encephalitis
* Poliomyelitis
* Diarrhoeal Diseases—Guidelines for the diagnosis of an outbreak.

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